The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte JONATHAN L.TILLY and RICHARD N. KOLESNICK

Appeal No. 2006-0076
Application No. 09/503,852

ON BRIEF

Before ADAMS, GRIMES and LINCK, Administrative Patent Judges.

LINCK, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of pending claims 1, 5-12, 17, 18, 20-23, 32 and 74. Brief at 20-22.

Claim 1 is representative and is the only independent claim in the application. All of the claims stand or fall together. See Brief at 9. Claim 1 reads as follows:

1. A method of treating a female reproductive system by administering to a female patient a composition comprising sphingosine-1-phosphate in an amount sufficient to inhibit apoptosis induced by an artificial insult, wherein said administration is *in vivo* or *ex vivo*, and wherein said artificial insult is a chemotherapeutic drug or radiation.

The prior art references relied upon by the Examiner are as follows:

Perez et al. ("Perez"), "Apoptosis-Associated Signaling Pathways Are Required For Chemotherapy-Mediated Female Germ Cell Destruction," Nature Medicine, Vol. 3, No. 11, pp. 1228-1232 (Nov. 1997).

Spiegel	5,712,262	Jan. 27, 1998
Igarashi et al. ("Igarashi")	5,877,167	Mar. 2, 1999

Claims 1, 5-12, 17, 18, 20-23, 32 and 74 stand rejected under 35 U.S.C. §103(a), based on Perez et al. in view of Spiegel and further in view of Igarashi.

We reverse.

Background

"Female gonads house a finite number of meiotically-arrested germ cells (oocytes) enclosed within primordial follicles that serve as the stockpile of eggs released at ovulation at each menstrual cycle . . . . Once depleted, the ovarian germ cell pool cannot be replenished. Thus, exposure of women to a wide spectrum of agents that damage the ovary, such as chemotherapeutic agents and radiotherapy, generally leads to premature menopause and irreversible sterility." Specification at 1(citations omitted).

"Apoptotic cell death plays a fundamental role in normal germ cell endowment and follicular dynamics in the ovary. Cell fate in the ovary is likely dependent on the actions of several proteins recently identified as key determinants of cell survival or death." *Id.* at 2 (citations omitted). A number of these proteins have been identified, including ceramide, a "recently identified lipid second messenger associated with cell death signaling . . . implicated in the induction of apoptosis in the ovary." *Id.* (citations omitted). According to the specification, at the time the application was filed, it was "known that ceramide can also be metabolized via ceramidase to sphingosine, which is then phosphorylated by sphingosine kinase to generate

sphingosine-1-phosphate (SIP)." *Id.* at 3 (citations omitted).

"In some cell types, SIP can effectively counterbalance stress-kinase activation and apoptosis induced by membrane-permeant ceramide analogs or external stressors known to work through elevations in intracellular ceramide levels. Therefore, a rheostat model has been proposed in which cell fate is controlled by shifts in the balance between ceramide and SIP levels." *Id.* However, according to the specification, "the physiologic importance of ceramide, and that of sphingomyelin hydrolysis as a whole, in activating developmental or homeostatic paradigms of apoptosis have recently been questioned by some investigators." *Id.* (citations omitted).

The specification notes that cell lineage will be "an important issue to consider based on observations that p53, a classic signaling molecule for cancer therapy-induced tumor cell destruction . . . , is completely dispensable for oocyte death initiated by cancer therapy." *Id.* at 4. Thus, according to the specification, "little is known regarding the mechanisms responsible for female germ cell destruction. Recently, it has been shown that female mouse oocytes undergo a type of cell death, referred to as apoptosis, when exposed *in vitro* to a prototypical anti-cancer drug (doxorubicin [DXR] . . .). Moreover, it is shown that culture of mouse oocytes *in vitro* with sphingosine-1-phosphate protected the oocytes from death induced by subsequent doxorubicin exposure." *Id.* However, "protection was only tested *in vitro* with only a single drug, and thus *in vivo* application remained questionable." *Id.*

The specification describes a number of *in vitro, in vivo* and *ex vivo* experiments to show the relationship between SIP and a decrease in oocyte apoptosis. *Id.* at 21-28. Based on the

results of these experiments, *inter alia*, the following conclusions are drawn:

(1) "[S]phingomyelin hydrolysis is a key event in generating death signals in the developing female germline" (Table 1 and Fig. 1); (2) "the rate of germ cell apoptosis is significantly attenuated in ASMase-deficient fetal ovaries cultured in parallel" with wild-type fetal ovaries (Fig. 2A); (3) "sphingomyelin hydrolysis, as opposed to ceramide synthesis, is important for generating ceramide as a death signal" (Fig. 2B); (4) "in support of the rheostat model, the reduced incidence of germ cell apoptosis conveyed by ASMase-deficiency is recapitulated by culturing wild-type fetal ovaries with increasing concentrations of SIP" (Fig. 2B); and (5) "in vivo administration of SIP two hours prior to irradiation resulted in a significant and dose-dependent preservation of the germ cell reserve, with complete protection of the quiescent (primordial) and growing (primary, preantral) follicle populations in ovaries" compared to "[n]early complete destruction" in "vehicle-treated ovaries" (Fig. 4). *Id.*

Discussion

Claim 1, the broadest claim on appeal, is directed to a method of treating a female reproductive system with a specific compound, sphingosine-1-phosphate, in an amount sufficient to inhibit cell death (apoptosis) caused by a chemotherapeutic drug or radiation.

The Scope and Content of the Prior Art

The Examiner relies upon three references to support his rejection under section 103(a). Tilly, one of the named inventors, co-authored the primary reference, Perez. Perez discloses *in vitro* "pretreatment of oocytes with sphingosine-1-phosphate (SP), an endogenous downstream inhibitor of ceramide-promoted intracellular signaling" resulting in oocyte survival in the presence

of a chemotherapeutic drug (doxorubicin). Perez at 1229. The authors conclude:

> female germ cells exposed to a widely used chemotherapeutic drug initiate apoptosis as a consequence of the activation of several death effector signaling pathways probably involving ceramide, Bax and caspases, but not p53. . . . Despite the significant advances made by this study in defining the biochemical and genetic pathways involved in oocyte destruction following exposure to anticancer drugs, future long-term studies are required to confirm that inhibiting germ cell apoptosis will preserve ovarian function. Nevertheless, these data provide a strong impetus for our current efforts to manipulate death effector pathways in oocytes, in vivo, as a potential means to overcome infertility associated with cancer treatment. [*Id.* at 1231.]

The second cited reference, Spiegel, discloses a "method of delaying programmed cell death by administration of a programmed cell death-delaying effective amount of sphingosine-1-phosphate." Speigel at col. 1, lines 57-60. Spiegel is focused on degenerative diseases such as ischemic stroke and aging. Intracellular levels of ceramide in human promyelocytic HL-60 cells and U937 monoblastic leukemia cells were increased to show increased apoptosis. The disclosed *in vitro* experiments suggest that exposure to sphingosine-1-phosphate prevents induced apoptosis in both cell lines. *Id.* at col. 3, lines 10-17. None of the experiments was performed *in vivo,* or using oocytes.

Igarashi, the final reference relied upon by the Examiner, adds little to Perez and Spiegel. It discloses the application of sphingosine-1-phosphate "for controlling cell motility and treating various disorders characterized by abnormal cell proliferation." Igarashi at col. 1, lines 65-67. While inhibition of chemoinvasion is one object of Igarashi's work, chemoinvasion "was measured by the ability of tumor cells . . . to migrate . . . during a prolonged incubation period" and does not appear to relate to apoptosis caused by chemotherapeutic drugs. *Id.* at col. 12, lines 26-29. Experiments were conducted *in vitro* on B16/F1 and B16/F10 mouse melanoma cells and human

fibrosarcoma cells. *Id.* at col. 5, lines 59-62. There is no disclosure regarding inhibition of cell apoptosis or impact on oocytes.

With respect to Spiegel and Igarashi, Appellants argue that these references "have absolutely nothing to do with oocytes or female reproduction" and should not be combined with Perez. Brief at 17 (emphasis in original). While these references do not disclose treatment of oocytes or applications to female reproduction, we do not agree with Appellants that they should not be combined with Perez, given their teachings with regard to sphingosine-1-phosphate. Clearly one skill in the art would be aware of references disclosing the use of this particular lipid in treating other infirmities. Nevertheless, these two references add little, if anything, to the teachings of Perez. They clearly do not bridge the gap between *in vitro* experiments and those conducted *in vivo* or *ex vivo*.

The Level of Skill in the Art

The level of skill in the art is not challenged and is reflected in the prior art references and other related art available at the time the invention was made.

The Differences Between the Claimed Invention and the Prior Art

Appellants distinguish Perez based on the claim language "by administering to a female patient" and "wherein administration is *in vivo* or *ex vivo*." Brief at 11-12. More specifically, Appellants have identified the differences between the claimed invention and the prior art as follows:

> The present claims are directed to methods of "treating a female reproductive system by administering to a female patient . . . sphingosine-1-phosphate . . . wherein said administration is *in vivo* or *ex vivo* . . . ." These methods do not encompass treating isolated oocytes in vitro, without returning

> the oocytes to the body. All of these methods require the *in vivo* or *ex vivo* use of sphingosine-1-phosphate to treat the female patient (as opposed to merely isolated cells). Moreover, the present claims are directed to treatments that are given in response to insults that occur *in vivo* rather than *in vitro.*
>
> Perez is directed only to the *in vitro* use of sphingosine-1-phosphate (i.e., administered to isolated oocytes) and not its *in vivo* or *ex vivo* use. The use of sphingosine-1-phosphate in Perez is limited to use in conjunction with *in vitro* insults. Spiegel and Igarashi are not directed to treating the female reproductive system in any manner [Brief at 12-13].

We agree with Appellants' characterization of the differences between the claimed invention and the prior art. Thus, we turn to the decisive question: Based on these differences and the record before us, should the Examiner's rejection be sustained?

Patentability of the Claimed Invention Under 35 U.S.C. § 103(a)

In order to hold that the claimed invention would have been obvious to one of ordinary skill in the art, we must decide whether that skilled artisan would have had a reasonable expectation of success at the time the application was filed. *See, e.g., In re Vaeck*, 947 F.2d 488, 493, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). Motivation to try to protect oocytes from insult *in vivo* or *ex vivo* is not sufficient. *See, e.g., Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 1208-09, 18 USPQ2d 1016, 1022-23 (Fed. Cir. 1991); *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1599 (Fed. Cir. 1988).

Appellants argue:

> Perez alone cannot provide a reasonable expectation of success for the claimed invention because:
>
> • Perez is directed to an *in vitro* method (administering sphingosine-1-phosphate to isolated oocytes) to protect against an *in vitro* insult (administering doxorubicin to isolated oocytes).
> •The present claims do not encompass *in vitro* methods to protect against *in*

vitro insults.

• Perez contains explicit statements of doubt as to whether Perez's *in vitro* results with isolated oocytes can be extrapolated to *in vivo* treatment of the female reproductive system as in the present claims. For example, Perez made it clear that *in vitro* effects are not predictive of *in vivo* successes such as preserving ovarian function. See page 1231, sentence bridging left and right columns:

> Despite the significant advances made by this study in defining the biochemical and genetic pathways involved in oocyte destruction following exposure to anticancer drugs, future long-term studies are required to confirm that inhibiting germ cell apoptosis will preserve ovarian function. [emphasis added by Appellants]

Perez also taught that in *vivo* treatments of the female reproductive system *(e.g.* preserving its fertility) require an effect not just on oocytes, but also on the follicles that support oocytes. See page 1230, lines 6-7: "[F]ertility preservation would require maintenance of the entire follicle and not solely the oocyte." Perez, page 1230, col. 1, lines 6-7. Perez contains no demonstration of the effects of sphingosine-1-phosphate on follicles and thus cannot provide a reasonable expectation of success for treatments that depend on effects on follicles.

The follicle is a structure found in ovaries that is formed by the physical interaction of oocytes and granulosa cells and is necessary for the growth, maturation, and survival of oocytes. Oocytes degenerate and die fairly quickly if not supported by the somatic cells found within follicles. For example, oocytes can survive for months (in mice) to years (in humans) when enclosed within follicles, but only for 24-48 hours once removed and placed in vitro. The *in vitro* results of Perez completely ignore the possibility of damage to other (non-oocyte) cells, such as either follicular granulosa cells or microvascular endothelial cells (blood supply), playing a role in oocyte depletion and ovarian failure caused by anti-cancer treatments.

It is clear that providing protection against radiation damage *in vivo* requires protecting granulosa cells as well as oocytes. Therefore, studies such as Perez, which shed no light on the ability of sphingosine-1-phosphate to protect granulosa cells from radiation damage, cannot provide a reasonable expectation of success for the practice of the claimed invention.

Moreover, the work described in Perez was conducted solely *in vitro*

> using fully mature (metaphase 11) oocytes obtained after superovulating female mice with exogenous gonadotropins (see page 1231, right column, under "Methods"). These fully mature oocytes are not comparable in terms of radiation sensitivity to the immature (germinal vesicle-stage) oocytes contained within the resting (primordial) and early growing (primary) follicles found *in vivo*. It is these immature oocytes that are the target population of the claimed invention. This target population of oocytes is not comparable to the oocytes of Perez because immature oocytes are much more sensitive to, and thus preferentially destroyed by, radiation *in vivo* [Brief at 14-17 (emphasis in orginal)].
>
> The Examiner responds:
>
> Perez is directed to female sterility resulting from exposure of oocytes from the reproductive system to doxorubicin, a chemotherapy drug. While the exposure is in vitro, the conditions for the exposure are made to simulate an in vivo environment as closely as possible. . . . How effective in vivo or whether it is effective in vivo is not considered an issue to be determined by this office. The reference shows what is known in the art. ... Spiegel teaches the use of sphingosine-1-phosphate to treat degenerative diseases. . . . Degenerative diseases encompass many diseases, including those that may affect the reproductive system. Igarashi et al teach[] the treatment of cells by administering sphingosine-1-phosphate. The treatment is not limited to a particular part of a mammal and therefore encompasses the reproductive system of a female. Both Spiegel, column 2, lines 3-27, and Igarashi et al, column 8, lines 4-20, teach in vivo administration of sphingosine-1-phosphate to mammals. . . .
>
> While Perez et al contain[] no demonstration of the effects of sphingosine-1-phosphate on follicles, applicants' claim 1 is not so limited. . . . Perez, Igarashi et al, and Spiegel all teach about an abnormality in relation to cellular function in mammals and the effect of administering sphingosine-1-phosphate. Applicants' claims to the female reproductive system encompass cellular function. [Answer at 3-6.]

The Examiner's arguments miss the point. First, while it is true the Office does not determine effectiveness of *in vivo* administration, the teachings and data in the application support Appellants' *in vivo* claims. As we understand this case, the Examiner has not challenged these teachings and data. Second, the fact that treatment of degenerative diseases "may affect the

reproductive system" or that a disclosed treatment is "not limited to a particular part of a mammal" does not address the main issue before us. That question is, would a skilled artisan have a reasonable expectation of success when attempting *in vivo* treatment? Third, while claim 1 is not limited to "the effects of sphingosine-1-phosphate on follicles," the significance of this lack of teaching in Perez goes to the reasonable expectation of success. Finally, as to Examiner's statement that both Spiegel and Igarashi "teach *in vivo* administration of" SIP to mammals, both references merely describe broad methods of administration and do not provide any evidence that such administration was successfully conducted. While it *may* be routine in the art to determine an effective form of administration and an effective dosage, descriptions such as those in Spiegel and Igarashi do not establish that one of ordinary skill in the art could apply the *in vitro* teachings of the prior art to an "*in vivo* or *ex vivo*" application with a reasonable expectation of success.

We agree with the Examiner that "in medicine virtually all drug therapies initially begin with in vitro studies" and "[s]uccess with in vitro studies would suggest efficacy in vivo." Answer at 5. However, we believe these facts merely support an "obvious to try" conclusion, not the conclusion necessary for us to affirm the rejection.

Conclusion

We find no evidence in any of the cited references that SIP had been successfully used *in vivo* or *ex vivo* prior to the filing of Appellants' application. While Perez may have provided a strong impetus to conduct *in vivo* experiments, based on the record before us, we conclude the early research described in Perez would not be sufficient to provide a reasonable expectation of success when doing so. Thus, given the state of and unpredictability in this art, we conclude that

the pending claims would not have been obvious under 35 U.S.C. § 103.

REVERSED

DONALD E. ADAMS
Administrative Patent Judge

ERIC B. GRIMES
Administrative Patent Judge

NANCY J. LINCK
Administrative Patent Judge

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